Steroid concentrations in rabbit oviducal fluid during oestrus and pseudopregnancy

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Summary. The concentrations of progesterone and oestradiol in the oviducal fluid during oestrus and pseudopregnancy were measured. Progesterone concentrations ranged from 0.55 ± 0.17 ng/ml during oestrus to a maximum of 2.86 ± 0.82 ng/ml on Day 12 of pseudopregnancy. Serum progesterone concentrations were similar to those found in oviduct fluid during oestrus, but by Day 12 serum levels had risen to 14.13 ± 1.97 ng/ml. Daily oviducal fluid oestradiol values ranged from 48.3 ± 6.4 pg/ml to 119.7 ± 23.6 pg/ml and were similar to serum concentrations.

Introduction


There have been numerous studies measuring the steroid concentrations in the serum of rabbits before and after ovulation and one report of the progesterone content of uterine flushings (Challis, Davies & Ryan, 1973; Fuchs & Beling, 1974; Thau & Lanman, 1975; Fowler, Johnson, Walters & Pratt, 1976; Spilman & Wilks, 1976; Waterson & Mills, 1976; Wu, Blasco, Flickinger & Mikhail, 1977a). There have been no reports, however, of the levels of steroids in rabbit oviducal fluid. Since the steroids in oviducal fluids could affect the reproductive processes occurring there, it is important to know what concentrations of oestrogen and progesterone the gametes and embryos encounter in the oviduct. This study therefore reports the measurement of progesterone and oestradiol in the oviducal fluid and peripheral serum of pseudopregnant rabbits.

Materials and Methods

Oviducal fluid was obtained daily from pseudopregnant rabbits by the intra-abdominal flask technique of Hamner & Williams (1965). Mature, New Zealand White does (4–4.5 kg) were induced to ovulate by i.v. injection of 100 i.u. hCG. Oviducal fluid from oestrous animals was
collected for 2 days before the induction of pseudopregnancy. All fluids were frozen until they were used for steroid determinations. Arterial blood was taken daily from the ears of another group of pseudopregnant animals for the measurement of serum steroid levels.

Progesterone and oestradiol-17β were measured using reagent kits from New England Nuclear (Boston, Massachusetts). The progesterone antiserum cross-reaction was 0-6% with 20α-dihydroprogesterone and 4-3% with 17α-hydroxyprogesterone. The cross-reaction of the oestradiol antiserum was 50% with oestrone and 25% with oestradiol-17α. After ether extraction, the steroids of interest were separated from contaminating substances by Sephadex LH-20 chromatography. The recovery of labelled progesterone and oestradiol ranged from 57 to 79% and 64 to 89%, respectively. Validation studies used a pool of oviducal fluid from ovariectomized rabbits not being treated with oestrogen (Stone, Richardson, Hamner & Oliphant, 1977) and serum from ovariectomized rabbits. When 1-0 ng progesterone/ml or 50 pg oestradiol/ml were added to oviducal fluid, 1-31 ± 0-15 (s.e.m.) mg/ml and 49-2 ± 8-9 (s.e.m.) pg/ml, respectively, were recovered. Measurements of serum of ovariectomized rabbits to which had been added 1-0 or 14-0 ng progesterone/ml or 50 pg oestradiol/ml gave recoveries of 1-34 ± 0-16 (s.e.m.) ng/ml and 15-28 ± 2-69 (s.e.m.) pg/ml for progesterone and 65-0 ± 4-8 (s.e.m.) pg/ml for oestradiol. The progesterone assay has a sensitivity of 15 pg/tube; the sensitivity for oestradiol is 10 pg/tube. The intra-assay coefficients of variation were 8-1% for progesterone and 4-2% for oestradiol; the inter-assay coefficients of variation were 7-0% for progesterone and 12-6% for oestradiol. These were determined by replicate assays of control serum. Water blanks were also run with each assay.

Differences in steroid concentrations in oviducal fluid or serum amongst the days of pseudopregnancy were analysed by using a one-way analysis of variance and Duncan’s multiple range test for which \( P < 0-05 \) was considered significant. Differences between oviducal fluid and serum steroid concentrations on each day were determined by a \( t \) test for two means for which \( P < 0-004 \) was accepted as significant.

Results

The volumes of oviducal fluid collected for steroid analysis and the progesterone and oestradiol values for these fluids and serum are shown in Table 1. Showing a decrease to half the oestrous volume between Days 4 and 16 of pseudopregnancy, the oviducal fluid secretion pattern is similar to that reported previously by several authors (Mastroianni & Wallach, 1961; Hamner, 1973; Oliphant, Bowling, Eng, Keen & Randall, 1978). There were two peaks of progesterone, a small peak on Day 2 which had declined to oestrous levels by Day 4, and a larger peak on Day 12. By Day 20 the progesterone had returned to nearly preovulatory levels. Only the values on Days 4 and 20 did not differ significantly from those at oestrus \( (P < 0-05) \). The oestradiol concentrations in the oviducal fluid appeared to rise to a peak on Day 14, but there were no significant differences among any of the days \( (P < 0-05) \). The increasing progesterone concentration during pseudopregnancy is not solely the result of the concomitant decrease in oviducal fluid volume since the amount measured, before correction for volume, also increased. By contrast, the oestradiol content of the fluids remained relatively constant throughout pseudopregnancy, indicating that the increased levels were probably a reflection of the decrease in fluid volume.

The small peak of progesterone on Day 2 was not detectable in the peripheral serum; serum progesterone levels rose steadily to a peak on Day 10, returning to nearly oestrous levels by Day 20. The serum progesterone concentrations on Days 4–16 were significantly higher than those of oestrus and early and late pseudopregnancy \( (P < 0-05) \). Serum oestradiol values fluctuated throughout pseudopregnancy: the values during oestrus and on Day 4 were significantly higher than those of the other days of the cycle \( (P < 0-05) \).
Table 1. Volumes and progesterone and oestradiol concentrations in rabbit oviducal fluid and serum

<table>
<thead>
<tr>
<th>Day</th>
<th>Oviduct fluid vol. (ml/day)</th>
<th>Progesterone (ng/ml)</th>
<th>Oestradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 12)</td>
<td>Oviducal fluid</td>
<td>Serum (n = 6)</td>
</tr>
<tr>
<td>Oc</td>
<td>1.2 ± 0.1</td>
<td>0.55 ± 0.17(10)</td>
<td>0.49 ± 0.09</td>
</tr>
<tr>
<td>2</td>
<td>1.0 ± 0.1</td>
<td>1.58 ± 0.26(11)</td>
<td>2.56 ± 0.33</td>
</tr>
<tr>
<td>4</td>
<td>0.7 ± 0.06</td>
<td>0.83 ± 0.08(10)</td>
<td>6.37 ± 1.11</td>
</tr>
<tr>
<td>6</td>
<td>0.7 ± 0.04</td>
<td>1.58 ± 0.17(12)</td>
<td>10.60 ± 1.05</td>
</tr>
<tr>
<td>8</td>
<td>0.7 ± 0.04</td>
<td>1.89 ± 0.18(12)</td>
<td>14.01 ± 0.73</td>
</tr>
<tr>
<td>10</td>
<td>0.7 ± 0.06</td>
<td>1.72 ± 0.20(12)</td>
<td>16.87 ± 1.46</td>
</tr>
<tr>
<td>12</td>
<td>0.6 ± 0.04</td>
<td>2.86 ± 0.82(8)</td>
<td>14.13 ± 1.97</td>
</tr>
<tr>
<td>14</td>
<td>0.6 ± 0.05</td>
<td>2.23 ± 0.39(11)</td>
<td>14.98 ± 1.41</td>
</tr>
<tr>
<td>16</td>
<td>0.7 ± 0.05</td>
<td>1.79 ± 0.31(10)</td>
<td>7.21 ± 1.70</td>
</tr>
<tr>
<td>18</td>
<td>0.8 ± 0.1</td>
<td>1.75 ± 0.54(10)</td>
<td>2.75 ± 0.55</td>
</tr>
<tr>
<td>20</td>
<td>0.9 ± 0.07</td>
<td>0.95 ± 0.16(12)</td>
<td>1.63 ± 0.32</td>
</tr>
</tbody>
</table>

Oc = oestrus.

Values are mean ± s.e.m. for the no. of samples indicated in parentheses.

Values significantly different from those in serum samples on the same day are indicated: *P < 0.004. Values for progesterone on Day 4 and oestradiol on Days 8, 12 and 14 reached only P < 0.01.

The oviducal fluid:serum ratios are also shown in Table 1. Serum progesterone concentrations were significantly higher than those of oviducal fluid on Days 4–14 (P < 0.004). Conversely, for oestradiol there appeared to be a definite trend toward higher oviducal fluid concentrations on Days 6–14 but the small number of samples and large individual variations prevented detection of significant differences at the conservative significance level employed (P < 0.004).

Discussion

The peripheral serum levels of progesterone observed in this study are similar to those reported previously for pregnant animals (Challis et al., 1973; Fuchs & Beling, 1974; Thau & Lanman, 1975); but are slightly higher than those reported for pseudopregnant animals (Fuchs & Beling, 1974; Thau & Lanman, 1975; Spilman & Wilks, 1976). The few studies of serum oestriol levels do not report values for the same days as the present investigation. However, the range of values in the current study is similar to that reported by Wu et al. (1977a) for pseudopregnant animals and Challis et al. (1973) for pregnant rabbits. Waterson & Mills (1976) report higher values during the first 24 h of pregnancy, and the concentrations observed by Spilman & Wilks (1976) are lower than those reported here. The small amount of oestriol in the serum is also consistent with the relatively low ovarian secretion rates reported by Hilliard & Eaton (1971) and by Spilman et al. (1978).

The only other report of steroid levels in oviducal fluids is that of Wu, Mastroianni & Mikhail (1977b) who studied fluids from monkeys. Due to procedural differences that report and the present results cannot be compared directly. However, the range of progesterone and oestriol concentrations reported for monkey oviducal fluid is similar to that measured in rabbit oviducal fluid.

Because the fluid collection technique excludes ova and embryos from the oviducal fluid, any contribution they might make to the steroid levels is not represented in these measurements. Steroid concentrations in rabbit follicular fluid are relatively high (Bahr, 1978). The amount of follicular fluid that remains trapped in the egg coatings has not been estimated, however, and the amount of steroid derived from this source is unknown. Most reports of steroid production by early embryos have only measured levels in 5-day or older blastocysts (Dickmann, Dey & Gupta, 1975; Borland, Erickson & Ducibella, 1977; Fujimoto & Sundaram, 1978). Since
embryos of this age have usually entered the uterus, it is unlikely that they make a significant addition to the oviducal fluid steroid levels. Although Dickmann et al. (1975) demonstrated 3β-hydroxysteroid dehydrogenase activity in embryos as early as 48 h post coitum, there are no reports of substantial amounts of steroids being produced by these early stage embryos. The steroid concentrations reported here, then, represent only steroids transferred from the circulation. However, the potential contribution by ova and embryos may be so negligible that the concentrations measured in this study may be the physiological levels in the normal oviduct.

The oviducal fluid: serum ratios for progesterone and oestradiol raise some questions concerning the factors that might be regulating the oviducal fluid steroid concentrations. In a limited study Waldham & Dickson (1976) reported that blood flow to the oviduct decreases between Days 6 and 15 of pregnancy. It is possible that this reduction of blood flow, during the time when serum progesterone concentrations are rising, is related to the lower oviducal fluid volumes observed and limits the amount of steroid that reaches the oviduct. Other factors that could be involved include steroid interactions with tissue receptors and conversion to metabolites by the epithelium. El-Banna & Sacher (1977) reported that the oestradiol binding capacity of the rabbit oviduct decreases 96 h post coitum and in response to exogenous progesterone administration. In addition, using secretory endometrium, Gurpide, Tseng & Gusberg (1977) observed similar changes in oestradiol receptor levels, and showed an increase in oestradiol-17β dehydrogenase activity (an enzyme that converts oestradiol to oestrone). The oviducal fluid steroid concentrations may also be influenced by the availability of steroid binding proteins in the oviduct. Wu et al. (1977b) reported that 46-9% of the progesterone and 64-4% of the oestradiol in monkey oviducal fluid was in the free form. Oliphant et al. (1978) reported that the serum albumin concentration in rabbit oviducal fluid was only 13% of that in serum. Likewise, the oviduct produces only a small amount of blastokinin (uteroglobin) in response to progesterone stimulation (Feigelson, Noske, Goswami & Kay, 1977).

This investigation did not attempt to determine the mechanism of steroid accumulation in oviducal fluid. Further research will be necessary to determine what, if any, relationships exist among blood flow, fluid volume, steroid-tissue interactions, and oviducal fluid steroid binding proteins which function to maintain the observed steroid concentrations.

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References


Chang, M.C. (1958) Capacitation of rabbit spermatozoa in the uterus with special reference to the


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